REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
13-08-2012	Final Report	15-May-2009 - 14-Feb-2012

4. TITLE AND SUBTITLE
Carotenoid antenna binding and function in retinal proteins

5a. CONTRACT NUMBER
W911NF-09-1-0243

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS
J. K. Lanyi, S. P. Balashov

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of California - Irvine
Office of Research Administration
The Regents of the University of California
Irvine, CA 92697 -7600

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
56107-LS.16

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
Xanthorhodopsin, a proton pump from the eubacterium Salinibacter ruber, is a unique dual chromophore system that contains, in addition to retinal, the carotenoid salinixanthin as a light-harvesting antenna. The key factors affecting binding and function of the antenna were established and examined. The first is the binding of the carotenoid ring near the retinal ring. Substitution of the small glycine with bulky tryptophan in this site eliminates binding. The second factor is the 4-keto group in the carotenoid ring. A close analogue of salinixanthin,

15. SUBJECT TERMS
excitation energy transfer, carotenoid antenna, light-driven proton pump, retinal protein

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sergei Balashov
UU	UU	UU	UU		19b. TELEPHONE NUMBER: 949-824-2720

Report Title

Carotenoid antenna binding and function in retinal proteins

ABSTRACT

Xanthorhodopsin, a proton pump from the eubacterium Salinibacter ruber, is a unique dual chromophore system that contains, in addition to retinal, the carotenoid salinixanthin as a light-harvesting antenna. The key factors affecting binding and function of the antenna were established and examined. The first is the binding of the carotenoid ring near the retinal ring. Substitution of the small glycine with bulky tryptophan in this site eliminates binding. The second factor is the 4-keto group in the carotenoid ring. A close analogue of salinixanthin, salinixanthol, in which the 4-keto group (C=O) is reduced to hydroxyl (C-OH), does not bind. Third, the binding site itself. Another protein capable of carotenoid binding was found. Gloeobacter rhodopsin, was shown to bind salinixanthin and echinenone but not beta-carotene or salinixanthol. Using femtosecond absorption spectroscopy, a large transient electrochromic shift of the salinixanthin band was detected 150 fs after excitation of the retinal chromophore. It indicates that excitation of retinal is accompanied by charge separation and a strong electrostatic field. It partially remains in the first photoproduct K. Thermal conversion of the latter involves at least two substates which were characterized with FTIR spectroscopy. The kinetics of photocycle and proton transport by xanthorhodopsin was examined.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
2012/07/27 2(	15	A. B. Rubin, M. P. Kirpichnikov, J. K. Lanyi, S. P. Balashov, L. E. Petrovskaya, E. P. Lukashev, E. S. Imasheva, A. K. Dioumaev, J. M. Wang, S. V. Sychev, D. A. Dolgikh. Aspartate–Histidine Interaction in the Retinal Schiff Base Counterion of the Light-Driven Proton Pump of, Biochemistry, (07 2012): 5748. doi: 10.1021/bi300409m
2011/10/31 1(	13	Václav Šlouf, Sergei P. Balashov, Janos K. Lanyi, Tõnu Pullerits, Tomáš Polívka. Carotenoid response to retinal excitation and photoisomerization dynamics in xanthorhodopsin, Chemical Physics Letters, (11 2011): 96. doi: 10.1016/j.cplett.2011.09.062
2011/08/30 1(	12	Sergei P. Balashov, Eleonora S. Imasheva, Jennifer M. Wang, Janos K. Lanyi. Removal and Reconstitution of the Carotenoid Antenna of Xanthorhodopsin, The Journal of Membrane Biology, (11 2010): 0. doi: 10.1007/s00232-010-9322-x
2011/07/12 1(	10	J.K.Lanyi, S.P.Balashov. XANTHORHODOPSIN. In HALOPHILES AND HYPERSALINE ENVIRONMENTS (eds. A. Ventosa, A. Oren, Y. Ma). Springer Verlag, p. 319-340. 2011., , (07 2011): . doi:
2010/12/31 2(	9	E. S. Imasheva, S. P. Balashov, J. M. Wang, J. K. Lanyi. Paper "Removal and Reconstitution of the Carotenoid Antenna of Xanthorhodopsin", Journal of Membrane Biology, (11 2010): . doi:
2010/11/19 1(	8	Balashov, S. P., E. S. Imasheva, A. R. Choi, K-H. Jung, S. Liaaen-Jensen. J. K. Lanyi. Reprint of the paper "Reconstitution of Gloeobacter Rhodopsin with Echinenone: Role of the 4-keto Group" by Balashov et al. (2010)Biochemistry 49, 9792-9799 , Biochemistry, (10 2010): . doi:
2010/07/08 1(	3	A. K. Dioumaev, J. M. Wang, and J. K. Lanyi.. Reprint (pdf file) of the paper “Low-Temperature FTIR Study of Multiple K Intermediates in the Photocycles of Bacteriorhodopsin and Xanthorhodopsin” by A. K. Dioumaev, J. M. Wang, and J. K. Lanyi. J. Phys. Chem. B, 2010, 114, 2920–2931 , Journal of Physical Chemistry B, (10 2009): . doi:
2009/11/23 1(	2	E. Imasheva, S. Balashov, A. Choi, K. Jung, J. Lanyi. Reconstitution of Gloeobacter violaceus rhodopsin with a light-harvesting carotenoid antenna, Biochemistry 48, 10948-10955 (2009), Biochemistry, (10 2009): . doi:

TOTAL: 8

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper	
2012/05/09 15	14	Sergei P. Balashov,, Lada E. Petrovskaya, , Evgeniy P. Lukashev, , Eleonora S. Imasheva, , Andrei K. Dioumaev, , Jennifer M. Wang, , Sergey V. Sychev, , Dmitriy A. Dolgikh,, Andrew B. Rubin, , Mikhail P. Kirpichnikov,, Janos K. Lanyi‡. Aspartate-Histidine Interaction in the Retinal Schiff Base Counterion of the Light-Driven Proton Pump of Exiguobacterium sibiricum, Biochemistry (03 2012)
2011/07/16 23	11	V. Šlouf, S. P. Balashov, J. K. Lanyi, T. Pullerits, T. Polívka. Manuscript: Carotenoid response to retinal excitation and photoisomerization dynamics in xanthorhodopsin, Chemical Physics Letters (07 2011)
2010/07/30 14	7	E.S. Imasheva, S. P. Balashov, J. M. Wang, J. K. Lanyi. Removal and Reconstitution of the Carotenoid Antenna of Xanthorhodopsin, The Journal of Membrane Biology (07 2010)
2010/07/29 15	6	S. P. Balashov, E. S. Imasheva, Ah Rheum Choi, Kwang-Hwan Jung, J. K. Lanyi. Reconstitution of Gloeobacter Rhodopsin with Echinenone: Role of the 4-keto Group, Biochemistry (07 2010)
2010/07/27 15	5	J. K. Lanyi, S. P. Balashov. Xanthorhodopsin, Book: "Halophiles and Hypersaline Environments", edited by A. Ventosa, A. Oren and Y. Ma (07 2010)
2009/09/09 15	1	E. Imasheva, S. Balashov, A. Choi, K-H. Jung, J. Lanyi. Reconstitution of Gloeobacter violaceus Rhodopsin with a Light-Harvesting Carotenoid Antenna" and supporting information , (09 2009)

TOTAL: **6**

Number of Manuscripts:

Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
S.P.Balashov	0.20	
J.K.Lanyi	0.05	
FTE Equivalent:	**0.25**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
E.S.Imasheva	0.20
J.Wang	0.20
FTE Equivalent:	**0.40**
Total Number:	**2**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

The following questions were addressed:

- Can the carotenoid antenna of xanthorhodopsin be removed and reconstituted?
- Which groups (atoms) of the carotenoid are crucial for its binding and energy transfer?
- Which residues of the protein are important for antenna binding?
- Are there other retinal proteins that bind carotenoids and use them for light-harvesting?
- Can carotenoid serve as a sensor for the intramolecular processes in energy conversion?
- Does the carotenoid undergo changes during the excitation of retinal, during the reactions of the photocycle and upon laser damage of the retinal protein?
- How do light-induced proton release and uptake correlate with photocycle reactions?
- Do the distinctively different structural features of xanthorhodopsin (compared to bacteriorhodopsin) correlate with their different mechanisms for proton release?
- Are these features common to other eubacterial proton pumps (e.g., proteorhodopsin, gloeobacter rhodopsin, ESR).

Summary of the most important results:

1. Removal and reconstitution of the carotenoid antenna of xanthorhodopsin.

Salinixanthin, a C40-carotenoid, serves as a light-harvesting antenna in the retinal-based proton pump xanthorhodopsin of Salinibacter ruber. This study shows that the carotenoid could be removed by oxidation with ammonium persulfate, with little effect on the absorption spectrum of other chromophore, retinal, and on the kinetics of the photocycle of xanthorhodopsin. The carotenoid-free protein can be reconstituted with salinixanthin extracted from the cell membrane of S. ruber. This restores the vibronic structure of the absorption spectrum of the bound carotenoid, its chirality, and the excited-state energy transfer to retinal. Surprisingly, minor modification of salinixanthin, by reducing the C=O double bond of the 4-keto group in the ring to C-OH, suppresses carotenoid binding and eliminates the antenna function. This indicates that the presence of the 4-keto group is critical for carotenoid binding and efficient energy transfer. Published in: Imasheva, E. S., S. P. Balashov, J. M. Wang and J. K. Lanyi. 2011. Removal and reconstitution of the carotenoid antenna of xanthorhodopsin. J. Membrane Biol. 239, 95-104. DOI: 10.1007/s00232-010-9322-x.

2. Reconstitution of gloeobacter rhodopsin with salinixanthin from Salinibacter ruber: Evidence for existence of other retinal proteins with light-harvesting antenna.
It was demonstrated that salinixanthin can be reconstituted into the retinal protein from Gloeobacter violaceus expressed in E. coli. Reconstitution of gloeobacter rhodopsin with the carotenoid is accompanied by characteristic absorption changes from sharpening of carotenoid absorption bands and the appearance of CD bands similar to those observed for xanthorhodopsin that indicate immobilization and twist of the carotenoid in the binding site. As in xanthorhodopsin, the carotenoid functions as a light-harvesting antenna. The excitation spectrum for retinal fluorescence emission shows that ca. 36% of the energy absorbed by the carotenoid is transferred to the retinal. From excitation anisotropy, the angle between the two chromophores was estimated as ca. 50º, similar to that in xanthorhodopsin. The results indicate that gloeobacter rhodopsin binds salinixanthin in a similar way as xanthorhodopsin, and suggest that it might bind similar carotenoid also in vivo. In the crystallographic structure of xanthorhodopsin, the 4-keto-ring is in the space occupied by a tryptophan in bacteriorhodopsin, which is replaced by the smaller glycine in xanthorhodopsin and gloeobacter rhodopsin. Specific binding of the carotenoid and its light-harvesting function are eliminated by a single mutation of the gloeobacter protein that replaces this glycine with a tryptophan (in the G178W mutant). This indicates that the 4-keto-ring is critically involved in carotenoid binding, and suggests that a number of other recently identified retinal proteins, from a diverse group of organisms, could also contain carotenoid antenna since they carry the homologous glycine near the retinal. This study was published in Imasheva, E. S., S. P. Balashov, A. R. Choi, K.-H. Jung, J. K. Lanyi. 2009. Reconstitution of Gloeobacter violaceus rhodopsin with a light-harvesting carotenoid antenna. Biochemistry 48, 10948-10955. DOI: 10.1021/bi901552x.

3. Reconstitution of gloeobacter rhodopsin with echinenone: crucial role of the 4-keto Group.

In previous work (see point 2), salinixanthinwas reconstituted into gloeobacter rhodopsin expressed in E. coli. There is no salinixanthin in Gloeobacter violaceous, but a simpler carotenoid, echinenone, also with a 4-keto ring but lacking the acyl glycoside, is present in addition to beta-carotene and oscillol. Two questions were examined: do any of the native Gloeobacter carotenoids bind to gloeobacter rhodopsin, and does the 4-keto group of the carotenoid ring play a role in binding.
Beta-carotene did not bind to gloeobacter rhodopsin, but its 4-keto derivative, echinenone, did. It functions as a light-harvesting antenna. This indicates that the 4-keto group is critical for the carotenoid binding. Further evidence for this is that an analogue of salinixanthin in which the C=O of the 4-keto group is reduced to hydroxyl C-OH, does not bind and is not engaged in energy transfer. It is suggested that the conjugated 4-keto group allowed for the twisted conformation of the ring around C6-C7 bond, and probably engaged in interaction that locks the carotenoid in the binding site. Published in: Balashov, S. P., E. S. Imasheva, A. R. Choi, K.-H. Jung, S. Liaaen-Jensen and J. K. Lanyi. 2010. Reconstitution of Gloeobacter rhodopsin with echinenone: role of the 4-keto group. Biochemistry 49, 9792-9799. DOI: 10.1021/bi1014166

4. Primary reaction of xanthorhodopsin involves two consecutive K like states and perturbation of two water molecules. Comparison with bacteriorhodopsin.
With low-temperature FTIR spectroscopy at 80-180K it was shown that the bacteriorhodopsin (bR) and xanthorhodopsin (XR) photocycles include three distinct K-like bathochromic intermediates: bR (XR) -> K0-> KE-> KL->L. The bathoproduct K0 is the main component in the mixture formed at 80 K from J. It becomes thermally unstable above ~50 K in both proteins. At 80 K, both J-to-K0 and K0-to-KE transitions occur and, contrarily to long-standing belief, cryogenic trapping at 80 K does not produce a pure K state but a mixture of the two states, K0 and KE, with contributions from KE of ~15 and ~10% in the two retinal proteins, respectively. Raising the temperature leads to increasing conversion of K0 to KE. The two states coexist in the 80-140 K range in bacteriorhodopsin, and in the 80-190 K range in xanthorhodopsin. The KE state, is the same intermediate that was detected by time-resolved FTIR, on a time scale of hundreds of nanoseconds at ambient temperature into the KL state. Formation of the two consecutive K-like states in both proteins is accompanied by distortion of two different weakly bound water molecules: one in K0, the other in KE. This study was published in: Dioumaev, A. K., J. M. Wang and J. K. Lanyi. 2010. Low-Temperature FTIR Study of Multiple K States in the Photocycles of Bacteriorhodopsin and Xanthorhodopsin. J. Phys. Chem. B. 114:2920-2931. DOI: 10.1021/jp908698f.

5. A review summarizing features of xanthorhodopsin and its unique carotenoid antenna.

The recently solved crystal structure of xanthorhodopsin, the first for eubacterial proton pumps, reveals not only the location of the two chromophores but considerably different architecture of the protein compared to the archaeal bacteriorhodopsin. Steady state fluorescence and femtosecond absorption spectroscopy provided a wealth of information on the pathway of excitation energy transfer from carotenoid second excited state S2 to retinal S1 and properties of the excited states involved. Carotenoid reconstitution experiments revealed conditions for carotenoid binding. Studies of the photocycle and proton transfer indicate that in xanthorhodopsin the proton release follows proton uptake, similar to proteorhodopsin but in the opposite sequence as in bacteriorhodopsin. These features correlate with structural differences in the design of xanthorhodopsin and bacteriorhodopsin, and suggest different means of proton transfer, especially proton release at the extracellular side in eubacterial proton pumps. Published in: Lanyi, J. K. and S. P. Balashov. 2011. Xanthorhodopsin. In “Halophiles and Hypersoline environments” (eds. A. Ventosa, A. Oren, Y. Ma). Springer Verlag, p. 319-340. (Book Chapter). DOI: 10.1007/978-3-642-20198-1_17

6. Laser-induced damage of xanthorhodopsin. First characterization of the photoproducts.

Laser-induced irreversible damage of xanthorhodopsin with 532 nm 6 ns flashes was found to occur starting at a threshold pulse intensity of ca 10 mJ/cm2. It results in formation of two stable photoproducts. One is a red shifted species that indicates neutralization of the counterion. The second product accumulates at higher doses and absorbs at 350 nm. This short wavelength absorption suggests deprotonation of the Schiff base. The absorption spectrum of the carotenoid antenna does not change significantly when native cell membranes were used in the experiments. This indicates that the damaged retinal chromophore remains attached to the protein since removal of the retinal from the binding site results in large changes in the bound salinixanthin that looses fine structure of its absorption spectrum.

7. Response of carotenoid antenna of xanthorhodopsin to retinal excitation and photoisomerization.

Excitation of the retinal chromophore of xanthorhodopsin (XR) induced a blue shift of the absorption band of the carotenoid salinixanthin, which is bound to the protein in vicinity of retinal and functions as a light-harvesting antenna. The shift was interpreted as electrochromic response to the strong transient electrostatic field of ca. 2.8 MV.cm-1 produced by transition of retinal to the excited state. The blue shift decays with the decay of the excited state of retinal but small part of it remains in the primary photoproduct K. The life time of the retinal excited state in XR decayed in biphasic manner with time constants 0.7 ps (70%) and 3 ps (30%), substantially slower than in bacteriorhodopsin, 0.4 ps (91%) and 2.1 ps (9%). Analysis of the carotenoid spectral shifts indicate that an internal field of 1.6 MV.cm-1 is present in the protein in the vicinity of the chromophores in the ground state. Published in a paper: Šlouf, V., S. P. Balashov, J. K. Lanyi, T. Polívka. 2011. Carotenoid response to retinal excitation and photoisomerization dynamics in xanthorhodopsin. Chem. Phys. Letters 516, 96-101. DOI: 10.1016/j.cplett.2011.09.062

8. The kinetics of light-induced proton release and proton uptake was studied with pH sensitive dyes, pyranine at pH 7.2 and thymol blue at pH 9. At both pH the uptake occurred first and followed the decay of the M intermediate, whereas proton release was at the end of the photocycle with time constant ca. 100 ms at pH 7.2. This is similar to proteorhodopsin but different from bacteriorhodopsin where release occurs first. Correlation of proton transfer steps with photocycle reactions and carotenoid absorption changes was established.

9. Structural differences between xanthorhodopsin and bacteriorhodopsin, and their implications for proton release in eubacterial rhodopsins was examined. The crystallographic structure of xanthorhodopsin indicates that in this eubacterial

light-driven proton pump the extensive extracellular hydrogen-bonded network observed in bacteriorhodopsin is absent. It is replaced by a deep water-filled cleft that reaches into the protein nearly as far as the retinal Schiff base. In xanthorhodopsin, and in eubacterial rhodopsins in general, of the two glutamic acid residues in bacteriorhodopsin that participate in proton release (Glu194, Glu204), only one is conserved, and at a location too far removed to be affected by protonation of the Schiff base counter-ion, which is now a histidine-aspartate complex rather than an aspartate. As a consequence, proton release is delayed until the last step of the photocycle, as in proteorhodopsin, and at that step it must utilize different residues to conduct the protons to the bulk. Is there a xanthorhodopsin-like cleft in other eubacterial proton pumps? This intriguing question was addressed experimentally. Bacteriorhodopsin, proteorhodopsin, and gloeobacter rhodopsin residues at several strategic locations on their extracellular surface and in the putative proton release channel were replaced with cysteine, and tested their reactivities with DTNB, the water soluble Edman-reagent for SH groups. The residues were selected for their strongly different accessibilities to water in bacteriorhodopsin and xanthorhodopsin. The results indicate that in the eubacterial proteins tested access followed the predictions from xanthorhodopsin structure, rather than from bacteriorhodopsin. From these results one can conclude that the structure of the extracellular surface, and therefore the means available for proton release, are fundamentally different in the archaeal and the eubacterial proton transport rhodopsins.

10. Asp-His interaction in proton pump from Exiguobacterium sibiricum homologous to xanthorodopsin. One of the distinctive features of eubacterial retinal based proton pumps, xanthorhodopsin, proteorhodopsins, and others, is hydrogen bonding of the key aspartate residue, the counterion to the retinal Schiff base, to a histidine. Properties of the recently found eubacterium proton pump from Exiguobacterium sibiricum (named ESR) expressed in E. coli, especially features that depend on Asp-His interaction, the protonation state of the key aspartate, Asp85, and its ability to accept proton from the Schiff base during the photocycle were examined. Proton pumping by liposomes and E. coli cells containing ESR occurs in a broad pH range above pH 4.5. Large light-induced pH changes indicate that ESR is a potent proton pump. Replacement of His57 with methionine or asparagine strongly affects the pH dependent properties of ESR. In the H57M mutant a dramatic decrease in the quantum yield of chromophore fluorescence emission and a 45 nm blue shift of the absorption maximum upon raising the pH from 5 to 8 indicates deprotonation of the counterion with a pKa of 6.3, which is also the pKa at which the M intermediate is observed in the photocycle of the protein solubilized in detergent (DDM). This is in contrast with the wild type protein, in which the same experiments show that the major fraction of Asp85 is deprotonated at pH > 3 and that it protonates only at low pH, with a pKa of 2.3. The M intermediate in the wild type photocycle accumulates only at high pH, with an apparent pKa of 9 from deprotonation of a residue interacting with Asp85, presumably His57. In liposomes reconstituted with ESR the pKas for M formation and spectral shifts are 2-3 pH units lower than in DDM. The distinctively different pH dependencies of the protonation of Asp85 and the accumulation of the M intermediate in the wild type protein vs. the H57M mutant indicate that there is strong Asp-His interaction, which substantially lowers the pKa of Asp85 by stabilizing its deprotonated state. Described in a paper Balashov, S.P. et al., 2012. Aspartate-Histidine Interaction in the Retinal Schiff Base Counterion of the Light-Driven Proton Pump of Exiguobacterium sibiricum. Biochemistry 51, 5748-5762. dx.doi.org/10.1021/bi300409m.

Papers credited to the ARO W911NF09-1-0243 Grant:

1. Imasheva, E. S., S. P. Balashov, A. R. Choi, K.-H. Jung, J. K. Lanyi. 2009. Reconstitution of Gloeobacter violaceus rhodopsin with a light-harvesting carotenoid antenna. Biochemistry 48, 10948-10955. DOI: 10.1021/bi901552x.

2. Dioumaev, A. K., J. M. Wang and J. K. Lanyi. 2010. Low-Temperature FTIR Study of Multiple K States in the Photocycles of Bacteriorhodopsin and Xanthorhodopsin. J. Phys. Chem. B. 114, 2920-2931. DOI: 10.1021/jp908698f

3. Balashov, S. P., E. S. Imasheva, A. R. Choi, K.-H. Jung, S. Liaaen-Jensen and J. K. Lanyi. 2010. Reconstitution of Gloeobacter rhodopsin with echinenone: role of the 4-keto group. Biochemistry 49, 9792-9799. DOI: 10.1021/bi1014166

4. Imasheva, E. S., S. P. Balashov, J. M. Wang and J. K. Lanyi. 2011. Removal and reconstitution of the carotenoid antenna of xanthorhodopsin. J. Membrane Biol. 239, 95-104. DOI: 10.1007/s00232-010-9322-x.

5. Lanyi, J. K. and S. P. Balashov. 2011. Xanthorhodopsin. In “Halophiles and Hypersoline environments” (eds. A. Ventosa, A. Oren, Y. Ma). Springer Verlag, p. 319-340. (Book Chapter). DOI: 10.1007/978-3-642-20198-1_17

6. Šlouf, V., S. P. Balashov, J. K. Lanyi, T. Polívka. 2011. Carotenoid response to retinal excitation and photoisomerization dynamics in xanthorhodopsin. Chem. Phys. Letters 516, 96-101. DOI: 10.1016/j.cplett.2011.09.062

7. Balashov, S.P., L.E. Petrovskaya, E.P. Lukashev, E.S. Imasheva, A.K. Dioumaev, J.M. Wang, S.V. Sychev, D.D. Dolgikh, A.B. Rubin, M.P. Kirpichnikov, Mikhail; and J.K. Lanyi 2012. Aspartate-Histidine Interaction in the Retinal Schiff Base Counterion of the Light-Driven Proton Pump of Exiguobacterium sibiricum. Biochemistry 51, 5748-5762. dx.doi.org/10.1021/bi300409m

Abstracts at the Conferences:

S. P. Balashov, E. S. Imasheva, A. R. Choi, K-H. Jung, J. K. Lanyi. Light-harvesting Carotenoid Antennae of Retinal Proteins. 14th International Conference on Retinal Proteins. UCSC, Porter College, Santa Cruz, CA, August 2-10, 2010.
J.K.Lanyi, J.M.Wang and S.P.Balashov. Structural Divergence between Archaeal and Eubacterial Proton Transport Rhodopsins. 14th International Conference on Retinal Proteins. UCSC, Porter College, Santa Cruz, CA, August 2-10, 2010.

Presentations at conferences, but not published in conference proceedings

1. S.P.Balashov. Xanthorhodopsin: excitation energy transfer and interaction between carotenoid antenna and retinal. 15th International Congress on Photobiology. June 18-23, 2009, Dusseldorf, Germany.

2. J.K.Lanyi. Eubacterial rhodopsins extend the range of structural requirements for proton transport. 15th International Congress on Photobiology. June 18-23, 2009, Dusseldorf, Germany.

3. J.K.Lanyi and S.P.Balashov. Interaction of antenna carotenoid and retinal in the light-driven pumps of Salinibacter ruber and Gloeobacter violaceus. International Conference "Halophiles 2010" June 29 - July 3, 2010, Beijing, China.

Technology Transfer